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<b>(54) Title:</b> USE OF AGONISTS OR ANTAGONISTS OF MIP-3A IN THERAPY <b>(57) Abstract</b> <p>Agonists or antagonists of MIP-3<math>\alpha</math>, and various methods of use in dermatological and related applications are provided. In particular, the method makes use of fact that the MIP-3<math>\alpha</math> chemokine is specifically capable of inducing migration of a skin cell subset.</p>		

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## USE OF AGONISTS OR ANTAGONISTS OF MIP-3A IN THERAPY

## 5 Field of the Invention

The invention relates generally to methods of using various chemokine related compositions, more particularly, to methods of treating skin diseases or conditions associated with misregulation of the chemokine MIP-3 $\alpha$ , a ligand for the CCR6 chemokine receptor.

## BACKGROUND

The immune system consists of a wide range of distinct cell types, each with important roles to play. See Paul (ed. 15 1997) Fundamental Immunology 4th ed., Raven Press, New York. The lymphocytes occupy central stage because they are the cells that determine the specificity of immunity, and it is their response that orchestrates the effector limbs of the immune system. Two broad classes of lymphocytes are 20 recognized: the B lymphocytes, which are precursors of antibody secreting cells, and the T (thymus-dependent) lymphocytes. T lymphocytes express important regulatory functions, such as the ability to help or inhibit the development of specific types of immune response, including 25 antibody production and increased microbicidal activity of macrophages. Other T lymphocytes are involved in direct effector functions, such as the lysis of virus infected-cells or certain neoplastic cells.

The chemokines are a large and diverse superfamily of 30 proteins. The superfamily is subdivided into two classical branches, based upon whether the first two cysteines in the chemokine motif are adjacent (termed the "C-C" branch), or spaced by an intervening residue ("C-X-C"). A more recently identified branch of chemokines lacks two cysteines in the 35 corresponding motif, and is represented by the chemokines known as lymphotactins. Another recently identified branch

has three intervening residues between the two cysteines, e.g., CX3C chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

- 5        Many factors have been identified which influence the differentiation process of precursor cells, or regulate the physiology or migration properties of specific cell types. These observations indicate that other factors exist whose functions in immune function were heretofore unrecognized.
- 10      These factors provide for biological activities whose spectra of effects may be distinct from known differentiation or activation factors. The absence of knowledge about the structural, biological, and physiological properties of the regulatory factors which regulate cell physiology in vivo
- 15      prevents the modulation of the effects of such factors. Thus, medical conditions where regulation of the development or physiology of relevant cells is required remain unmanageable.

## SUMMARY OF THE INVENTION

The present invention is based, in part, upon the surprising discovery that the MIP-3 $\alpha$  chemokine is expressed in inflamed skin cells. The chemokine is the ligand for the CCR6 receptor. See Greaves, et al. (1997) J. Expt'l Med. 186:837-844. Both the ligand and receptor are expressed at essentially undetectable levels in normal skin, while both are highly upregulated in inflamed skin.

The present invention provides methods of modulating migration of a cell within or to the skin of a mammal comprising administering to the mammal an effective amount of: an antagonist of MIP-3 $\alpha$ ; an agonist of MIP-3 $\alpha$ , and antagonist of CCR6; or an agonist of CCR6. Typically, the migration is within the skin; or may be chemotactic or chemokinetic. In preferred embodiments, the administering is systemic, local, topical, subcutaneous, intracutaneous, or transdermal. Often, the cell is a T cell, B cell, dendritic cell, or dendritic cell precursor. In other embodiments, the cell is a T cell, or moves into the dermal and/or epidermal layers of the skin.

In other embodiments, the administering is of an antagonist of MIP-3 $\alpha$ . Generally, the antagonist is selected from: a mutein of natural MIP-3 $\alpha$ ; an antibody which neutralizes MIP-3 $\alpha$ ; or an antibody which binds to CCR6. In various embodiments, the mammal is subject to a skin disease or condition, including one selected from cancer, cancer metastasis, skin transplant, or skin graft. Often, the antagonist is administered in combination with an antibiotic, antifungal, antiviral, or analgesic; or may be with an immune suppressive therapeutic, anti-inflammatory drug, growth factor, or immune adjuvant.

In another embodiment, the administering is with a primate MIP-3 $\alpha$ . Often, the modulating is attracting the cell, e.g., to a site of cutaneous lesion. The primate MIP-3 $\alpha$  may be administered in combination with an antibiotic, antifungal, antiviral, or analgesic; or with a vasodilator, growth factor, cytokine, anti-inflammatory drug, or immune adjuvant.

Alternatively, the invention provides a method of purifying a population of cells, the method comprising contacting the cells with MIP-3 $\alpha$ , thereby resulting in the identification of cells expressing a receptor for MIP-3 $\alpha$ . In  
5 certain embodiments, the receptor is CCR6, or the contacting results in specific migration of the cells to a site for purification, e.g., through pores of a membrane.

## DETAILED DESCRIPTION OF THE INVENTION

## Outline

- I. General
- 5 II. Chemokine Agonists and Antagonists
  - A. MIP-3 $\alpha$  and Variants
  - B. Antibodies
  - C. Other Molecules
- III. Immunoassays
- 10 IV. Uses

## I. General

The invention is based, in part, on the surprising discovery that the chemokine MIP-3 $\alpha$  has been implicated in roles in skin immunity. In particular, MIP-3 $\alpha$  has been identified as a ligand for the chemokine receptor designated CCR6. Both MIP-3 $\alpha$  and CCR6 expression are undetectable in normal skin, while both are highly upregulated in inflamed skin samples.

The skin consists of a surface layer of epithelium called the epidermis and an underlying layer of connective tissue called the dermis. Under the dermis is a layer which contains large amounts of adipose tissue, the hypodermis. The skin serves a variety of functions, and variations in the character of the dermis and epidermis occur according to functional demands. The appendages of the skin, hair, nails, and sweat and sebaceous glands, are such local specializations of the epidermis. Together, the skin and its appendages form the integument. See, e.g., Fitzpatrick, et al. (eds. 1993) Dermatology in General Medicine 4th ed., McGraw-Hill, NY; Bos (ed. 1989) Skin Immune System CRC Press, Boca Raton, FL; Callen (1996) General Practice Dermatology Appleton and Lange; Rook, et al. (eds. 1998) Textbook of Dermatology Blackwell; Habifor and Habie (1995) Clinical Dermatology: A Color Guide to Diagnosis and Therapy Mosby; and Grob (ed. 1997) Epidemiology, Causes and Prevention of Skin Diseases Blackwell.

The epidermis consists of many different cell types in various proportions. The most prevalent cell type is keratinocytes, which make up some 95% of the cells. Cells in the 1-2% range include melanocytes and Langerhans cells. The Langerhans cells are particularly important because they trap antigens that have penetrated the skin, and transport antigens to regional lymph nodes. A small population of  $\gamma\delta$  T cells can also reside in the epidermis.

The dermis varies in thickness in different regions of the body. It is tough, flexible, and highly elastic, and consists of a feltwork of collagen fibers with abundant elastic fibers. The connective tissue is arranged into deep reticular and superficial papillary layers.

The chemokines are a sub-family of chemoattractant cytokines that were classically characterized by their ability to mediate leukocyte trafficking or migration by binding to specific G-protein-linked seven transmembrane spanning receptors, or GPCRs. Chemokines are divided into four groups based on the primary sequence of the first two cysteines: the CXC, CC, C, and CX3C families. The CXC and C families are effective predominantly on neutrophils and lymphocytes, respectively. The CC chemokines are preferentially effective on macrophages, lymphocytes, and eosinophils.

The chemokine MIP-3 $\alpha$ , from human, mouse, and rat, has been described earlier. See, e.g., human, GenBank HSU77035; mouse, GenBank AF099052; rat, GenBank U90447; Li and Adams, WO 94-US9484; and Wilde, et al. WO 9616979; each of which is incorporated herein by reference for all purposes. The sequences are provided in Table 1.



Table 1: A primate, human, MIP-3 $\alpha$ , nucleic acid sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2). Coding sequence begins at about nucleotide 1 and ends at about 288; CC motif at amino acid residues 6-7. A signal sequence is indicated, but based upon related genes; slightly different processing may occur in different cell types.

5	atg tgc tgt acc aag agt ttg ctc ctg gct gct ttg atg tca gtg ctg	48
	Met Cys Cys Thr Lys Ser Leu Leu Leu Ala Ala Leu Met Ser Val Leu	
	-25 -20 -15	
10	cta ctc cac ctc tgc ggc gaa tca gaa gca gca agc aac ttt gac tgc	96
	Leu Leu His Leu Cys Gly Glu Ser Glu Ala Ala Ser Asn Phe Asp Cys	
	-10 -5 -1 1 5	
15	tgt ctt gga tac aca gac cgt att ctt cat cct aaa ttt att gtg ggc	144
	Cys Leu Gly Tyr Thr Asp Arg Ile Leu His Pro Lys Phe Ile Val Gly	
	10 15 20	
20	ttc aca cgg cag ctg gcc aat gaa ggc tgt gac atc aat gct atc atc	192
	Phe Thr Arg Gln Leu Ala Asn Glu Gly Cys Asp Ile Asn Ala Ile Ile	
	25 30 35	
25	ttt cac aca aag aaa aag ttg tct gtg tgc gca aat cca aaa cag act	240
	Phe His Thr Lys Lys Lys Leu Ser Val Cys Ala Asn Pro Lys Gln Thr	
	40 45 50	
30	tgg gtg aaa tat att gtg cgt ctc ctc agt aaa aaa gtc aag aac atg	288
	Trp Val Lys Tyr Ile Val Arg Leu Leu Ser Lys Lys Val Lys Asn Met	
	55 60 65 70	
	taa	291

Table 1 (continued): A murine, mouse, MIP-3 $\alpha$  chemokine nucleic acid sequence (SEQ ID NO: 3) and corresponding amino acid sequence (SEQ ID NO: 4). SignalP software predicts a cleavage between Ala(-1) and Ser1; but the actual cleavage may be on either side by a residue or so.

5	atg gcc tgc ggt ggc aag cgt ctg ctc ttc ctt gct ttg gca tgg gta	48
	Met Ala Cys Gly Gly Lys Arg Leu Leu Phe Leu Ala Leu Ala Trp Val	
	-25 -20 -15	
10	ctg ctg gct cac ctc tgc agc cag gca gaa gca agc aac tac gac tgt	96
	Leu Leu Ala His Leu Cys Ser Gln Ala Glu Ala Ser Asn Tyr Asp Cys	
	-10 -5 -1 1 5	
15	tgc ctc tcg tac ata cag acg cca ctt cct tcc aga gct att gtg ggt	144
	Cys Leu Ser Tyr Ile Gln Thr Pro Leu Pro Ser Arg Ala Ile Val Gly	
	10 15 20	
20	ttc aca aga cag atg gcc gat gaa gct tgt gac att aat gct atc atc	192
	Phe Thr Arg Gln Met Ala Asp Glu Ala Cys Asp Ile Asn Ala Ile Ile	
	25 30 35	
25	ttt cac acg aag aaa aga aaa tct gtg tgc gct gat cca aag cag aac	240
	Phe His Thr Lys Lys Arg Lys Ser Val Cys Ala Asp Pro Lys Gln Asn	
	40 45 50	
30	tgg gtg aaa agg gct gtg aac ctc ctc agc cta aga gtc aag aag atg	288
	Trp Val Lys Arg Ala Val Asn Leu Leu Ser Leu Arg Val Lys Lys Met	
	55 60 65	
30	taa	291

Table 1 (continued): A murine, rat, MIP-3 $\alpha$  chemokine nucleic acid sequence (SEQ ID NO: 5) and corresponding amino acid sequence (SEQ ID NO: 6). SignalP software predicts a cleavage between Ala(-1) and Ala1; but the actual cleavage may be on either side by a residue or so.

5	atg gcc tgc aag cat ctg ccc ttc ctg gct ttg gcg ggg gta ctg ctg	48
	Met Ala Cys Lys His Leu Pro Phe Leu Ala Leu Ala Gly Val Leu Leu	
	-25 -20 -15 -10	
10	gct tac ctc tgc agc cag tca gaa gca gca agc aac ttt gac tgc tgc	96
	Ala Tyr Leu Cys Ser Gln Ser Glu Ala Ala Ser Asn Phe Asp Cys Cys	
	-5 -1 1 5	
15	ctc acg tac aca aag aac gtg tat cat cat gcg aga aat ttt gtg ggt	144
	Leu Thr Tyr Thr Lys Asn Val Tyr His His Ala Arg Asn Phe Val Gly	
	10 15 20	
20	ttc aca aca cag atg gcc gac gaa gct tgt gac att aat gct atc atc	192
	Phe Thr Thr Gln Met Ala Asp Glu Ala Cys Asp Ile Asn Ala Ile Ile	
	25 30 35	
25	ttt cac ctg aag tcg aaa aga tcc gtg tgc gct gac cca aag cag atc	240
	Phe His Leu Lys Ser Lys Arg Ser Val Cys Ala Asp Pro Lys Gln Ile	
	40 45 50 55	
30	tgg gtg aaa agg att ttg cac ctc ctc agc cta aga acc aag aag atg	288
	Trp Val Lys Arg Ile Leu His Leu Leu Ser Leu Arg Thr Lys Lys Met	
	60 65 70	
30	taa	291

Table 2: Nucleotide sequence (5' to 3') of primate, human, chemokine receptor, CCR6, and the corresponding amino acid sequence (amino to carboxy), see SEQ ID NO: 7 and 8. Nucleotide 579 may be A, C, G, or T, and the codon may code for His or Gln.

5	atg ttt tgc act cca gtg aag att att ttg tgt cag tca ata ctt cat	48
	Met Phe Ser Thr Pro Val Lys Ile Ile Leu Cys Gln Ser Ile Leu His	
	1 5 10 15	
10	att act cag ttg att ctg aga tgt tac tgt gct cct tgc agg agg tca	96
	Ile Thr Gln Leu Ile Leu Arg Cys Tyr Cys Ala Pro Cys Arg Arg Ser	
	20 25 30	
15	ggc agt tct cca ggc tat ttg tac cga att gcc tac tcc ttg atc tgt	144
	Gly Ser Ser Pro Gly Tyr Leu Tyr Arg Ile Ala Tyr Ser Leu Ile Cys	
	35 40 45	
20	gtt ctt ggc ctc ctg ggg aat att ctg gtg gtg atc acc ttt gct ttt	192
	Val Leu Gly Leu Leu Gly Asn Ile Leu Val Val Ile Thr Phe Ala Phe	
	50 55 60	
25	tat aag aag gcc agg tct atg aca gac gtc tat ctc ttg aac atg gcc	240
	Tyr Lys Lys Ala Arg Ser Met Thr Asp Val Tyr Leu Leu Asn Met Ala	
	65 70 75 80	
30	att gca gac atc ctc ttt gtt ctt act ctc cca ttc tgg gca gtg agt	288
	Ile Ala Asp Ile Leu Phe Val Leu Thr Leu Pro Phe Trp Ala Val Ser	
	85 90 95	
35	cat gcc act ggt gcg tgg gtt ttc agc aat gcc acg tgc aag ttg cta	336
	His Ala Thr Gly Ala Trp Val Phe Ser Asn Ala Thr Cys Lys Leu Leu	
	100 105 110	
40	aaa ggc atc tat gcc atc aac ttt aac tgc ggg atg ctg ctc ctg act	384
	Lys Gly Ile Tyr Ala Ile Asn Phe Asn Cys Gly Met Leu Leu Leu Thr	
	115 120 125	
45	tgc att agc atg gac cgg tac atc gcc att gta cag gcg act aag tca	432
	Cys Ile Ser Met Asp Arg Tyr Ile Ala Ile Val Gln Ala Thr Lys Ser	
	130 135 140	
50	ttc cgg ctc cga tcc aga aca cta ccg cgc agc aaa atc atc tgc ctt	480
	Phe Arg Leu Arg Ser Arg Thr Leu Pro Arg Ser Lys Ile Ile Cys Leu	
	145 150 155 160	
55	gtt gtg tgg ggg ctg tca gtc atc atc tcc agc tca act ttt gtc ttc	528
	Val Val Trp Gly Leu Ser Val Ile Ile Ser Ser Ser Thr Phe Val Phe	
	165 170 175	
60	aac caa aaa tac aac acc caa ggc agc gat gtc tgt gaa ccc aag tac	576
	Asn Gln Lys Tyr Asn Thr Gln Gly Ser Asp Val Cys Glu Pro Lys Tyr	
	180 185 190	

Table 2 (continued):

5	can act gtc tcg gag ccc atc agg tgg aag ctg ctg atg ttg ggg ctt Xaa Thr Val Ser Glu Pro Ile Arg Trp Lys Leu Leu Met Leu Gly Leu	624
	195 200 205	
10	gag cta ctc ttt ggt ttc ttt atc cct ttg atg ttc atg ata ttt tgt Glu Leu Leu Phe Gly Phe Phe Ile Pro Leu Met Phe Met Ile Phe Cys	672
	210 215 220	
15	tac acg ttc att gtc aaa acc ttg gtg caa gct cag aat tct aaa agg Tyr Thr Phe Ile Val Lys Thr Leu Val Gln Ala Gln Asn Ser Lys Arg	720
	225 230 235 240	
20	cac aaa gcc atc cgt gta atc ata gct gtg gtg ctt gtg ttt ctg gct His Lys Ala Ile Arg Val Ile Ile Ala Val Val Leu Val Phe Leu Ala	768
	245 250 255	
25	tgt cag att cct cat aac atg gtc ctg ctt gtg acg gct gct aat ttg Cys Gln Ile Pro His Asn Met Val Leu Leu Val Thr Ala Ala Asn Leu	816
	260 265 270	
30	ggt aaa atg aac cga tcc tgc cag agc gaa aag cta att ggc tat acg Gly Lys Met Asn Arg Ser Cys Gln Ser Glu Lys Leu Ile Gly Tyr Thr	864
	275 280 285	
35	aaa act gtc aca gaa gtc ctg gct ttc ctg cac tgc tgc ctg aac cct Lys Thr Val Thr Glu Val Leu Ala Phe Leu His Cys Cys Leu Asn Pro	912
	290 295 300	
40	gtg ctc tac gct ttt att ggg cag aag ttc aga aac tac ttt ctg aag Val Leu Tyr Ala Phe Ile Gly Gln Lys Phe Arg Asn Tyr Phe Leu Lys	960
	305 310 315 320	
45	atc ttg aag gac ctg tgg tgt gtg aga agg aag tac aag tcc tca ggc Ile Leu Lys Asp Leu Trp Cys Val Arg Arg Lys Tyr Lys Ser Ser Gly	1008
	325 330 335	
50	ttc tcc tgt gcc ggg agg tac tca gaa aac att tct cgg cag acc agt Phe Ser Cys Ala Gly Arg Tyr Ser Glu Asn Ile Ser Arg Gln Thr Ser	1056
	340 345 350	
55	gag acc gca gat aac gac aat gcg tcg tcc ttc act atg tga Glu Thr Ala Asp Asn Asp Asn Ala Ser Ser Phe Thr Met	1098
	355 360 365	

In contrast to naive lymphocytes, memory/effector lymphocytes can access non-lymphoid effector sites and display restricted, often tissue-selective, migration behavior. This results in the presence of such lymphocytes in the peripheral  
5 tissues, e.g., outside of the lymphatic and blood volume.

Both human and mouse MIP-3 $\alpha$  are detected in lymph nodes, appendix, PBL, fetal liver, fetal lung, and various cell lines. See, e.g., Rossi, et al. (1997) J. Immunol. 158:1033-1036; Hieshima, et al. (1997) J. Biol. Chem. 272:5846-5853;  
10 Baba, et al. (1997) J. Biol. Chem. 272:14893-14898; and Imai, et al. (1997) J. Biol. Chem. 272:15036-15042. The expression in the Langerhans islets suggests a role in skin functions. The data is consistent with MIP-3 $\alpha$  as a product of activated monocytes, and is preferentially expressed in inflamed tissue.  
15 This distribution would suggest that MIP-3 $\alpha$  may have a role in attracting memory T cells, and skin dendritic cells (Langerhans cells) and their precursors. These results suggest an important role for MIP-3 $\alpha$  in recruitment of T cells and dendritic cells to peripheral cutaneous sites.

20 Chemokine receptors are members of the G protein coupled receptor family. See, e.g., Yoshie, et al. (1997) J. Leukoc. Biol. 62:634-644. CCR6 expression has been reported in Greaves, et al. (1997) J. Expt'l Med. 186:837-844; and Liao, et al. (1999) J. Immunol. 162:186-194. Northern blot data  
25 showed expression predominantly in the spleen, with lesser amounts in thymus, testis, small intestine, and peripheral blood. Additional transcripts were detected in spleen. Transcripts were not detected in the TF-1, Jurkat, MRC5, JY, and U937 cell lines. Message seems not to be abundantly  
30 expressed in the lymphoid lineage, particularly in, e.g., libraries made from cells made from dendritic cell cultures derived from cells selected on the basis of CD1a expression. Expression is lower in DC generated from monocytes.

Another study showed CCR6 was expressed on memory T  
35 cells, including most  $\alpha 4\beta 7$  memory cells and cutaneous lymphocyte-associated antigen expressing cells, and on B

cells. Chemotaxis of T cells to MIP-3 $\alpha$  was limited to memory cells. See Liao, et al. (1998) J. Immunol. 162:186-194. Antiserum detected CCR6 on CD34+ bone marrow derived dendritic cells.

5        Having identified the MIP-3 $\alpha$  as a skin related chemokine, it will find use in affecting medical abnormalities of the skin. Common skin disorders involving the immune system include psoriasis, skin cancers, carcinomas, inflammation, allergies, dermatitis, wound healing, infections (both  
10        microbial and parasitic), and many others. See, e.g., The Merck Manual, particularly the chapter on dermatologic disorders. These therapeutics may have useful effects on growth or health of appendages of the skin, including, e.g., hair, nails, and sweat and sebaceous glands.

15        Psoriasis is a chronic inflammatory skin disease that is associated with hyperplastic epidermal keratinocytes and infiltrating mononuclear cells, including T cells, neutrophils and macrophages. Because of this highly mixed inflammatory picture and the resulting complex interrelationships between  
20        these different cells, it has been very difficult to dissect the mechanisms that underlie the induction and progression of the disease.

      This view of psoriasis also implies that although dormant autoreactive T cells may pre-exist in susceptible individuals,  
25        an environmental stimulus is necessary to trigger disease induction. Others believe that the immune system plays only a minor modulatory role in the disease process and that hyperproliferation of keratinocytes is in fact the initiating event in a genetically susceptible host. Research into the  
30        pathogenesis of psoriasis has long been hindered by the lack of suitable animal models.

      There is growing data indicating that T cells and not keratinocytes are the primary pathogenic component in the disease. The observations herein provide evidence to support  
35        the concept that psoriasis-like conditions can indeed result from unregulated T cell responses.

Skin cancers such as basal cell and squamous cell carcinoma are among the most common malignancies. See, e.g., Miller and Maloney (eds. 1997) Cutaneous Oncology: Pathophysiology, Diagnosis, and Management Blackwell; Emmett and Orourke (1991) Malignant Skin Tumours Churchill Livingstone; Friedman (1990) Cancer of the Skin Saunders. Most of those tumors arise in sun exposed areas of the skin. Immune regulation or clearance of such tumors may depend upon function of the skin immune system. Cells which effect such may be compromised by local misregulation or suppression. The MIP-3 $\alpha$  or antagonists may break a temporary homeostasis which suppresses normal immune response, thereby leading to activation of proper regulatory and immune pathways.

Dermatitis is a superficial inflammation of the skin, characterized by vesicles (when acute), redness, edema, oozing, crusting, scaling, and/or itching. See, e.g., Lepoittevin (ed. 1998) Allergic Contact Dermatitis: The Molecular Basis Springer-Verlag; Rietschel and Fowler (eds. 1995) Fisher's Contact Dermatitis Lippincott; and Rycroft, et al. (eds. 1994) Textbook of Contact Dermatitis Springer-Verlag. The term eczematous dermatitis is often used to refer to a vesicular dermatitis. Dermatitis may accompany various immune deficiency conditions or diseases, inborn metabolic disorders, or nutritional deficiency diseases. Certain of the symptoms of such conditions may be treated using the present invention.

Pruritus is a sensation that the patient attempts to relieve by scratching. See, e.g., Fleischer and Fleischer (1998) The Clinical Management of Itching: Therapeutic Protocols for Pruritus Parthenon. Many parasitic or infectious conditions may result in those symptoms, which conditions may be cleared by proper reactivation or suppression of immune functions in the skin. Likewise with various allergic or other immune reactions to exposure to various allergic or inflammatory antigens.



## II. Chemokine Agonists and Antagonists

Mammalian MIP-3 $\alpha$  chemokines were described previously in USSN 08/887,977, which describes various migratory assays. Various agonists and antagonists of the natural ligands can be produced. The migration assays may take advantage of the movement of cells through pores in membranes. Chemotaxis may be measured thereby. Alternatively, chemokinetic assays may be developed, which measure the induction of kinetic movement, not necessarily relative to a gradient, per se.

### 10 A. MIP-3 $\alpha$ and variants

MIP-3 $\alpha$  agonists will exhibit some or all of the signaling functions of MIP-3 $\alpha$ , e.g., binding, inducing a Ca<sup>++</sup> flux, and chemoattracting appropriate receptor bearing cells. Various mammalian MIP-3 $\alpha$  sequences may be evaluated to determine what residues are conserved across species, suggesting what residues may be changed without dramatic effects on biological activity. Alternatively, conservative substitutions are likely to retain biological activity, thus leading to variant forms of the chemokine which will retain agonist activity. Standard methods for screening mutant or variant MIP-3 $\alpha$  polypeptides will determine what sequences will be useful therapeutic agonists.

In addition, certain nucleic acid expression methods may be applied. For example, in skin graft contexts, it may be useful to transfect the grafts with nucleic acids which will be expressed, as appropriate. Various promoters may be operably linked to the gene, thereby allowing for regulated expression. Antisense constructs may prevent expression of the ligand or receptor.

Alternatively, antagonist activity may be tested or screened for. Tests for ability to antagonize chemoattractant activity can be developed using assays as described below. Various ligand homologs can be created which retain receptor binding capacity, but lack signaling capability, thus serving as competitive binding molecules. Small molecules may also be screened for ability to antagonize MIP-3 $\alpha$  function, e.g.,

chemoattraction, receptor binding, Ca++ flux, and other effects mediated by MIP-3 $\alpha$ . See generally Gilman, et al.

(eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; Remington's

5 Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn, each of which is incorporated herein by reference.

#### B. Antibodies

The present invention provides for the use of an antibody  
10 or binding composition which specifically binds to MIP-3 $\alpha$ , preferably mammalian, e.g., primate, human, cat, dog, rat, or mouse, and neutralizes the ability of the chemokine to mediate its signal. Antibodies can be raised to various MIP-3 $\alpha$   
15 proteins, including individual, polymorphic, allelic, strain, or species variants, and fragments thereof, either in their naturally occurring (full-length) forms or in their recombinant forms. Additionally, antibodies can be raised to MIP-3 $\alpha$  or polypeptides in both their native (or active) forms or in their inactive, e.g., denatured, forms, which may  
20 neutralize ligand capacity to mediate its signal. Antibodies may block the interaction of the ligand with its receptor.

Alternatively, receptor antagonists may be produced by making antibodies which bind to the receptor and block ligand binding. With the identification of the CCR6 as a receptor  
25 for the cytokine, antibodies to the receptor may be selected for those which block the binding of, or signaling induced by, ligand.

A number of immunogens may be selected to produce antibodies specifically reactive, or selective for binding,  
30 with MIP-3 $\alpha$  or CCR6 proteins. Recombinant protein is a preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein, from appropriate sources, e.g., primate, rodent, etc., may also be used either in pure or impure form. Synthetic peptides, made  
35 using the MIP-3 $\alpha$  or CCR6 protein sequences described herein, may also be used as an immunogen for the production of

antibodies. Recombinant protein can be expressed and purified in eukaryotic or prokaryotic cells as described, e.g., in Coligan, et al. (eds. 1995 and periodic supplements) Current Protocols in Protein Science John Wiley & Sons, New York, NY; 5 and Ausubel, et al (eds. 1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York, NY. Naturally folded or denatured material, perhaps expressed on cell surfaces, can be used, as appropriate, for producing antibodies. Either monoclonal or polyclonal 10 antibodies may be generated, e.g., for subsequent use in immunoassays to measure the protein, or for immunopurification methods.

Methods of producing polyclonal antibodies are well known to those of skill in the art. Typically, an immunogen, 15 preferably a purified protein, is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to, e.g., the MIP-3 $\alpha$ , protein or polypeptide of interest. For example, 20 when appropriately high titers of antibody to the immunogen are obtained, usually after repeated immunizations, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be performed, if desired. See, 25 e.g., Harlow and Lane Antibodies, A Laboratory Manual; or Coligan (ed.) Current Protocols in Immunology. Immunization can also be performed through other methods, e.g., DNA vector immunization. See, e.g., Wang, et al. (1997) Virology 228:278-284. Affinity purification, or absorptions, can be 30 used to select for desired specificity of binding.

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell. 35 See, Kohler and Milstein (1976) Eur. J. Immunol. 6:511-519. Alternative methods of immortalization include transformation

with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. See, e.g., Doyle, et al. (eds. 1994 and periodic supplements) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, New York, NY. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according, e.g., to the general protocol outlined by Huse, et al. (1989) Science 246:1275-1281.

Antibodies or binding compositions, including binding fragments and single chain versions, against predetermined fragments of MIP-3 $\alpha$  or CCR6 polypeptides can be raised by immunization of animals with conjugates of the fragments with carrier proteins as described above. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective MIP-3 $\alpha$  protein, or screened for capacity to block cell MIP-3 $\alpha$  mediated chemoattraction or chemokinetic activity. These monoclonal antibodies will usually bind with at least a  $K_D$  of about 1 mM, more usually at least about 300  $\mu$ M, typically at least about 10  $\mu$ M, more typically at least about 30  $\mu$ M, preferably at least about 10  $\mu$ M, and more preferably at least about 3  $\mu$ M or better.

In some instances, it is desirable to prepare monoclonal antibodies (mAbs) from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.). Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal

Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY; and particularly in Kohler and Milstein (1975) Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method

5 involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate  
10 individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic  
15 substance.

Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. See, e.g., Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science  
20 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and  
25 antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent  
30 moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see, Cabilly, U.S. Patent No.  
35 4,816,567; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA

86:10029-10033; or made in transgenic mice, see Mendez, et al. (1997) Nature Genetics 15:146-156.

Antibody binding compounds, including binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be useful as non-neutralizing binding compounds and can be coupled to toxins or radionuclides so that when the binding compound binds to the antigen, a cell expressing it, e.g., on its surface, is killed. Further, these binding compounds can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

#### C. Other Molecules

Antibodies are merely one form of specific binding compositions. Other binding compositions, which will often have similar uses, include molecules that bind with specificity to MIP-3 $\alpha$  receptor, e.g., CCR6, in a binding partner-binding partner fashion, an antibody-antigen interaction, or in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent, e.g., proteins which specifically associate with MIP-3 $\alpha$  receptor protein. The molecule may be a polymer, or chemical reagent. A functional analog may be a protein with structural modifications, or may be a structurally unrelated molecule, e.g., which has a molecular shape which interacts with the appropriate binding determinants. Application of, e.g., Systematic Evolution of Ligand by Exponential Enrichment (SELEX) technology, methods are available to select specific binding constructs for desired targets. See, e.g., Colas, et al. (1996) Nature 380:548-550; Cohen, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:14272-14277; Kolonin, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:14266-14271; Famulok, et al. (1998) Curr. Opin. Chem. Biol. 2:320-327; and Eaton, et al. (1997) Bioorg. Med. Chem. 5:1087-1096.

Drug screening using antibodies or MIP-3 $\alpha$  or fragments thereof can be performed to identify compounds having binding

affinity to MIP-3 $\alpha$ , or can block or simulate the natural interaction with ligand. Subsequent biological assays can then be utilized to determine if the compound has intrinsic blocking activity and is therefore an antagonist. Likewise, a compound having intrinsic stimulating activity can signal to the cells via the MIP-3 $\alpha$  pathway and is thus an agonist in that it simulates the activity of a ligand. Mutein antagonists may be developed which maintain receptor binding but lack signaling.

Structural studies of the ligands will lead to design of new variants, particularly analogs exhibiting agonist or antagonist properties on the receptor. This can be combined with previously described screening methods to isolate muteins exhibiting desired spectra of activities.

As receptor specific binding molecules are provided, also included are small molecules identified by screening procedures. In particular, it is well known in the art how to screen for small molecules which interfere, e.g., with ligand binding to the receptor, often by specific binding to the receptor and blocking of binding by natural ligand. See, e.g., meetings on High Throughput Screening, International Business Communications, Southborough, MA 01772-1749. Such molecules may compete with natural ligands, and selectively bind to the MIP-3 $\alpha$  or CCR6

### III. Immunoassays

Immunoassays are valuable in diagnosing a disease or disorder associated with MIP-3 $\alpha$  imbalance or pathology. Qualitative or quantitative measurement of a particular protein can be performed by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds. 1991) Basic and Clinical Immunology (7th ed.). Moreover, the immunoassays of the present invention can be performed in many configurations, which are reviewed extensively in, e.g., Maggio (ed. 1980) Enzyme Immunoassay CRC Press, Boca Raton, Florida; Tijan

(1985) "Practice and Theory of Enzyme Immunoassays,"  
Laboratory Techniques in Biochemistry and Molecular Biology,  
Elsevier Science Publishers B.V., Amsterdam; Harlow and Lane  
Antibodies: A Laboratory Manual, supra; Chan (ed. 1987)  
5 Immunoassay: A Practical Guide Academic Press, Orlando, FL;  
Price and Newman (eds. 1991) Principles and Practice of  
Immunoassays Stockton Press, NY; and Ngo (ed. 1988) Non-  
isotopic Immunoassays Plenum Press, NY.

10 In particular, the present invention provides various  
skin related diseases as conditions susceptible to analysis or  
diagnosis by evaluating MIP-3 $\alpha$  and/or CCR6. For example, the  
likelihood of skin rejection in a graft situation would be  
evaluated by the numbers or types of MIP-3 $\alpha$  or CCR6 bearing  
cells present. Prophylactic downregulation may be useful to  
15 prevent the recruitment of dermal T or NK cells. Response to  
various skin tumors may be evaluated by the presence or  
absence of MIP-3 $\alpha$  and/or CCR6 bearing cells.

Immunoassays for measurement of MIP-3 $\alpha$  proteins or  
peptides can be performed by a variety of methods known to  
20 those skilled in the art. In brief, immunoassays to measure  
the protein can be either competitive or noncompetitive  
binding assays. In competitive binding assays, the sample to  
be analyzed competes with a labeled analyte for specific  
binding sites on a capture agent bound to a solid surface.  
25 Preferably the capture agent is an antibody specifically  
reactive with MIP-3 $\alpha$  proteins produced as described above.  
The concentration of labeled analyte bound to the capture  
agent is inversely proportional to the amount of free analyte  
present in the sample.

30 In a competitive binding immunoassay, typically the MIP-  
3 $\alpha$  protein present in the sample competes with labeled protein  
for binding to a specific binding agent, e.g., an antibody  
specifically reactive with the MIP-3 $\alpha$  protein. The binding  
agent may be bound to a solid substrate or surface to effect  
35 separation of bound labeled protein from the unbound labeled  
protein. Alternately, the competitive binding assay may be



conducted in liquid phase and a variety of techniques known in the art may be used to separate the bound labeled protein from the unbound labeled protein. Following separation, the amount of bound labeled protein is determined. The amount of protein  
5 present in the sample is inversely proportional to the amount of labeled protein binding.

Alternatively, a homogeneous immunoassay may be performed in which a separation step is not needed. In these immunoassays, the label on the protein is altered by the  
10 binding of the protein to its specific binding agent. This alteration in the labeled protein results in a decrease or increase in the signal emitted by label, so that measurement of the label at the end of the immunoassay allows for detection or quantitation of the protein.

15 MIP-3 $\alpha$  proteins may also be determined by a variety of noncompetitive immunoassay methods. For example, a two-site, solid phase sandwich immunoassay may be used. In this type of assay, a binding agent for the protein, e.g., an antibody, is attached to a solid support. A second protein binding agent,  
20 which may also be an antibody, and which binds the protein at a different site, is labeled. After binding at both sites on the protein has occurred, the unbound labeled binding agent is removed and the amount of labeled binding agent bound to the solid phase is measured. The amount of labeled binding agent  
25 bound is directly proportional to the amount of protein in the sample.

Western blot analysis can be used to determine the presence of MIP-3 $\alpha$  or CCR6 proteins in a sample. Electrophoresis is carried out, for example, on a tissue  
30 sample suspected of containing the protein. Following electrophoresis to separate the proteins, and transfer of the proteins to a suitable solid support, e.g., a nitrocellulose filter, the solid support is incubated with an antibody reactive with the protein. This antibody may be labeled, or  
35 alternatively may be detected by subsequent incubation with a second labeled antibody that binds the primary antibody.

The immunoassay formats described above may employ labeled assay components. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels and methods may be used. Traditionally, a radioactive label incorporating  $^3\text{H}$ ,  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^{32}\text{P}$  was used. Non-radioactive labels include ligands which bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation. For a review of various labeling or signal producing systems which may be used, see U.S. Patent No. 4,391,904.

Antibodies reactive with a particular protein can also be measured by a variety of immunoassay methods. Thus modifications of the above procedures may be used to determine the amounts or affinities of various MIP-3 $\alpha$  or CCR6 antibodies or antibody preparations. For a review of immunological and immunoassay procedures applicable to the measurement of antibodies by immunoassay techniques, see, e.g., Stites and Terr (eds.) Basic and Clinical Immunology (7th ed.) supra; Maggio (ed.) Enzyme Immunoassay, supra; and Harlow and Lane Antibodies, A Laboratory Manual, supra.

Screens to evaluate the binding and activity of mAbs and binding compositions encompass a variety of methods. Binding can be assayed by detectably labeling the antibody or binding composition as described above. Cells responsive to MIP-3 $\alpha$  can be used to assay antibody or binding composition.

To evaluate MIP-3 $\alpha$  chemoattraction or chemokinetic ability, experimental animals, e.g., mice, are preferably used. Skin, e.g., Langerhans, cell counts are made prior to and at various time points after administration of a bolus of the candidate agonist or antagonist. Levels are analyzed in various samples, e.g., blood, serum, nasal or pulmonary lavages, or tissue biopsy staining. A successful depleting

mAb or binding composition will significantly lower the level of CCR6 bearing cells. Such may be at least about 10%, preferably at least about 20%, 30%, 50%, 70%, or more.

5 Evaluation of antibodies can be performed in other animals, e.g., humans using various methods. For example, blood samples are withdrawn from patients suffering from a skin related disease or disorder before and after treatment with a candidate mAb.

## IV. Uses

The exquisite tissue-selective homing of lymphocytes has long been appreciated as central for the control of systemic immune responses. Recent advances in the field support a model in which leukocyte homing is achieved by sequential engagement of differentially expressed and independently regulated vascular and leukocyte adhesion molecules, and signaling receptors and their ligands. Butcher and Picker (1996) Science 272:60-66. The observation that chemokines, a superfamily of small secreted proteins with G protein-coupled receptors (Baggiolini (1998) Nature 392:565-568) can attract leukocytes led to the hypothesis that chemokines provide key signals directing recruitment of T lymphocyte subsets into lymphoid and extra-lymphoid immune effector sites. The inflamed skin-specific expression of MIP-3 $\alpha$  and CCR6 suggests that such skin-specific chemokines selectively attract functional subsets of lymphocytes into the skin.

As such, the present invention provides means to purify desired skin cell subsets. The chemoattractive or chemokinetic effects on those cells can be the basis of purification methods. Methods exist for selective migration and recovery of cells to or from the chemokine, e.g., through porous membrane, or to various locations in a culture. Other methods exist to selectively separate cells of particular shapes from others. Alternatively, labeling can be used to FACS sort cells which specifically bind the chemokine. Populations of substantially homogeneous Langerhans or skin derived cells will have important utility in research or therapeutic environments.

While MIP-3 $\alpha$  is likely to have functional effects on CCR6 bearing subsets of cells, e.g., T and B cells and precursors, other cells which may also be responsive include dendritic cells or granulocytes, e.g., neutrophils and/or eosinophils, or their precursors. Effects on various cell types may be indirect, as well as direct. A statistically significant change in the numbers of cells will typically be at least

about 10%, preferably 20%, 30%, 50%, 70%, 90%, or more. Effects of greater than 100%, e.g., 130%, 150%, 2X, 3X, 5X, etc., will often be desired. The effects may be specific in causing chemotaxis to specific points, or may be chemokinetic, in inducing general movement of cells, but not necessarily in a specific direction, e.g., of concentration gradient.

The present invention will be useful in the treatment of medical conditions or diseases associated with immunological conditions of the skin. See, e.g., Bos (ed. 1990) Skin Immune System CRC Press, Boca Raton, FL; Fitzpatrick, et al. (eds. 1993) Dermatology in General Medicine (4th ed.) McGraw-Hill, NY; Rook, et al. (eds. 1998) Textbook of Dermatology Blackwell; Habifor and Habie (1995) Clinical Dermatology: A Color Guide to Diagnosis and Therapy Mosby; Grob (ed. 1997) Epidemiology, Causes and Prevention of Skin Diseases Blackwell; Frank, et al. (eds. 1995) Samter's Immunologic Diseases, 5th Ed., vols. I-II, Little, Brown and Co., Boston, MA; Coffman, et al (1989) Science 245:308-310; and Frick, et al. (1988) J. Allergy Clin. Immunol. 82:199-225. The agonists or antagonists described may be combined with other treatments of the medical conditions described herein, e.g., an antibiotic, antifungal, antiviral, immune suppressive therapeutic, immune adjuvant, analgesic, anti-inflammatory drug, growth factor, cytokine, vasodilator, or vasoconstrictor.

The CCR6 receptor appears to be preferentially expressed on CD4+ memory T cells. Its ligand, MIP-3 $\alpha$ , is an inflammatory chemokine expressed by cellular constituents of the skin, whose expression is inducible after stimulation with T cell-derived proinflammatory mediators such as IFN- $\gamma$  and IL-17. Thus, CD4+ memory T cell mediated skin conditions are therapeutic targets of the antagonists, e.g., psoriasis, atopic dermatitis, contact dermatitis, SLE, and lichen ruber planus.

Preferred combination therapies include the MIP-3 $\alpha$  reagent with various anti-inflammatory agents, such as

topical, transdermal, or systemic steroids or corticosteroids. Systemic, topical, transdermal, or systemic retinoid or retinoid-like compounds, or vitamin D analogs, may be administered with the MIP-3 $\alpha$  therapeutics. Alternatively,  
5 various forms of UV light may be used in combination with these therapeutics, e.g., ultraviolet A, ultraviolet B, or narrow bands of UVB.

For example, the MIP-3 $\alpha$  ligands would be expected to signal specifically to the cell types expressing their  
10 receptor. Thus, it will be possible to block signaling, e.g., to the T cell or B cell subsets, by reagents which block receptor signaling, e.g., antibodies to ligand, and small drug antagonists.

Standard immunological techniques are described, e.g., in  
15 Hertenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. These will allow use of the  
20 reagents for purifying cell subpopulations, etc.

To prepare pharmaceutical or sterile compositions including, e.g., MIP-3 $\alpha$ , the material is admixed with a pharmaceutically acceptable carrier or excipient which is preferably inert. Preparation of such pharmaceutical  
25 compositions is known in the art, see, e.g., Remington's Pharmaceutical Sciences and U.S. Pharmacopeia: National Formulary, Mack Publishing Company, Easton, PA (1984). Typically, therapeutic compositions are sterile. Alternatively, MIP-3 $\alpha$  antagonist compositions can be prepared.

30 Agonists, e.g., natural ligand, or antagonists, e.g., antibodies or binding compositions, are normally administered parenterally, preferably intravenously. Since such protein or peptide antagonists may be immunogenic they are preferably administered slowly, either by a conventional IV  
35 administration set or from a subcutaneous depot, e.g. as taught by Tomasi, et al., U.S. patent 4,732,863. However, as

a skin target, the administration may be topical, transdermal, intradermal, subcutaneous, or even systemic.

When administered parenterally the therapeutics will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. The antagonist may be administered in aqueous vehicles such as water, saline, or buffered vehicles with or without various additives and/or diluting agents. Alternatively, a suspension, such as a zinc suspension, can be prepared to include the peptide. Such a suspension can be useful for subcutaneous (SQ), intradermal (ID), or intramuscular (IM) injection. The proportion of therapeutic entity and additive can be varied over a broad range so long as both are present in effective amounts. The therapeutic is preferably formulated in purified form substantially free of aggregates, other proteins, endotoxins, and the like, at concentrations of about 5 to 30 mg/ml, preferably 10 to 20 mg/ml. Preferably, the endotoxin levels are less than 2.5 EU/ml. See, e.g., Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications 2d ed., Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets 2d ed., Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY; Fodor, et al. (1991) Science 251:767-773; Coligan (ed.) Current Protocols in Immunology; Hood, et al. Immunology Benjamin/Cummings; Paul (ed. 1997) Fundamental Immunology 4th ed., Academic Press; Parce, et al. (1989) Science 246:243-247; Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011; and Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York. Local, e.g., topical or transdermal, administration will often be particularly useful.

Selecting an administration regimen for a therapeutic agonist or antagonist depends on several factors, including the serum or tissue turnover rate of the therapeutic, the immunogenicity of the therapeutic, or the accessibility of the

target cells. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of therapeutic delivered depends in part on the

5 particular agonist or antagonist and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies is found in the literature on therapeutic uses, e.g. Bach et al., chapter 22, in Ferrone, et al. (eds. 1985) Handbook of Monoclonal Antibodies Nokes Publications, 10 Park Ridge, NJ; and Russell, pgs. 303-357, and Smith et al., pgs. 365-389, in Haber, et al. (eds. 1977) Antibodies in Human Diagnosis and Therapy Raven Press, New York, NY.

Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known in the art 15 to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Numbers of CCR6 20 bearing cells in defined samples might be important indicators of when an effective dose is reached. Preferably, an antibody or binding composition thereof that will be used is derived from the same species as the animal targeted for treatment, thereby minimizing a humoral response to the reagent.

25 The total weekly dose ranges for antibodies or fragments thereof, which specifically bind to MIP-3 $\alpha$ , range generally from about 1 ng, more generally from about 10 ng, typically from about 100 ng; more typically from about 1  $\mu$ g, more typically from about 10  $\mu$ g, preferably from about 100  $\mu$ g, and 30 more preferably from about 1 mg per kilogram body weight. Although higher amounts may be more efficacious, the lower doses typically will have fewer adverse effects. Generally the range will be less than 100 mg, preferably less than about 50 mg, and more preferably less than about 25 mg per kilogram 35 body weight.



The weekly dose ranges for antagonists, e.g., antibody, binding fragments, range from about 10  $\mu$ g, preferably at least about 50  $\mu$ g, and more preferably at least about 100  $\mu$ g per kilogram of body weight. Generally, the range will be less than about 1000  $\mu$ g, preferably less than about 500  $\mu$ g, and more preferably less than about 100  $\mu$ g per kilogram of body weight. Dosages are on a schedule which effects the desired treatment and can be periodic over shorter or longer term. In general, ranges will be from at least about 10  $\mu$ g to about 50 mg, preferably about 100  $\mu$ g to about 10 mg per kilogram body weight.

Other antagonists of the ligands, e.g., muteins, are also contemplated. Hourly dose ranges for muteins range from at least about 10  $\mu$ g, generally at least about 50  $\mu$ g, typically at least about 100  $\mu$ g, and preferably at least 500  $\mu$ g per hour. Generally the dosage will be less than about 100 mg, typically less than about 30 mg, preferably less than about 10 mg, and more preferably less than about 6 mg per hour. General ranges will be from at least about 1  $\mu$ g to about 1000  $\mu$ g, preferably about 10  $\mu$ g to about 500  $\mu$ g per hour.

In particular contexts, e.g., transplant or skin grafts, may involve the administration of the therapeutics in different forms. For example, in a skin graft, the tissue may be immersed in a sterile medium containing the therapeutic resulting in a prophylactic effect on cell migration soon after the graft is applied.

The present invention also provides for administration of MIP-3 $\alpha$  antibodies or binding compositions in combination with known therapies, e.g., steroids, particularly glucocorticoids, which alleviate the symptoms associated with excessive inflammatory responses. Daily dosages for glucocorticoids will range from at least about 1 mg, generally at least about 2 mg, and preferably at least about 5 mg per day. Generally, the dosage will be less than about 100 mg, typically less than about 50 mg, preferably less than about 20 mg, and more preferably at least about 10 mg per day. In general, the

ranges will be from at least about 1 mg to about 100 mg, preferably from about 2 mg to 50 mg per day.

The phrase "effective amount" means an amount sufficient to effect a desired response, or to ameliorate a symptom or sign of the skin condition. Typical mammalian hosts will include mice, rats, cats, dogs, and primates, including humans. An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route, and dose of administration and the severity of side effects. Preferably, the effect will result in a change in quantitation of at least about 10%, preferably at least 20%, 30%, 50%, 70%, or even 90% or more. When in combination, an effective amount is in ratio to a combination of components and the effect is not limited to individual components alone.

An effective amount of therapeutic will modulate the symptoms typically by at least about 10%; usually by at least about 20%; preferably at least about 30%; or more preferably at least about 50%. Alternatively, modulation of migration will mean that the migration or trafficking of various cell types is affected. Such will result in, e.g., statistically significant and quantifiable changes in the numbers of cells being affected. This may be an increase or decrease in the numbers of target cells being attracted within a time period or target area.

The present invention provides reagents which will find use in therapeutic applications as described elsewhere herein, e.g., in the general description for treating disorders associated with skin conditions. See, e.g., Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, NY; Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn; Langer (1990)

Science 249:1527-1533; and Merck Index, Merck & Co., Rahway, New Jersey.

Antibodies to MIP-3 $\alpha$  proteins may be used for the identification or sorting of cell populations expressing MIP-3 $\alpha$  protein, e.g., fibroblasts or Langerhans cells. Methods to sort such populations are well known in the art, see, e.g., Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY. Populations of cells expressing the MIP-3 $\alpha$  receptor, e.g., CCR6, can also be purified, e.g., using magnetic beads as described, e.g., in Bieva, et al. (1989) Exp. Hematol. 17:914-920; Hernebtub, et al. (1990) Bioconj. Chem. 1:411-418; Vaccaro (1990) Am. Biotechnol. Lab. 3:30.

Moreover, antisense nucleic acids may be used. For example, antisense polynucleotides against the ligand encoding nucleic acids may function in a manner like ligand antagonists, and antisense against the receptor may function like receptor antagonists. Thus, it may be possible to block the signaling through the pathway with antisense nucleic acids. Conversely, nucleic acids for the receptor may serve as agonists, increasing the numbers of receptor on the cell, thereby increasing cell sensitivity to ligand, and perhaps blocking the normal apoptotic signal described.

Using the assay methods described above, the antibodies or binding compositions are useful in diagnosing diseases states which result in skin disorders. Antibodies raised against a MIP-3 $\alpha$  or CCR6 protein will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens. Combinations of these signals may be also pursued.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

## EXAMPLES

## I. General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA; and Coligan, et al. (eds.) (1995 and periodic supplements) Current Protocols in Protein Science, John Wiley & Sons, New York, NY. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Hertenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and

Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163.

Lymphocyte migration assays are performed as previously described, e.g., in Bacon, et al. (1988) Br. J. Pharmacol. 95:966-974. Other trafficking assays are also available. See, e.g., Quidling-Järbrink, et al. (1995) Eur. J. Immunol. 25:322-327; Koch, et al. (1994) J. Clinical Investigation 93:921-928; and Antony, et al. (1993) J. Immunol. 151:7216-7223.

Alternatively, an activation assay or attraction assay is used. An appropriate cell type is selected, e.g., hematopoietic cells, myeloid (macrophages, neutrophils, polymorphonuclear cells, etc.) or lymphoid (T cell, B cell, or NK cells), neural cells (neurons, neuroglia, oligodendrocytes, astrocytes, etc.), or stem cells, e.g., progenitor cells which differentiate to other cell types, e.g., gut crypt cells and undifferentiated cell types.

Chemokines may also be assayed for activity in hemopoietic assays as described, e.g., by H. Broxmeyer. See Bellido, et al. (1995) J. Clinical Investigation 95:2886-2895; and Jilka, et al. (1995) Expt'l Hematology 23:500-506. They may be assayed for angiogenic activities as described, e.g., by Streiter, et al. (1992) Am. J. Pathol. 141:1279-1284. Or for a role in inflammation. See, e.g., Wakefield, et al. (1996) J. Surgical Res. 64:26-31.

Other assays will include those which have been demonstrated with other chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.  $Ca^{2+}$  flux upon chemokine stimulation is measured according to the published procedure described in Bacon, et al. (1995) J. Immunol. 154:3654-3666.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY;

and Robinson, et al. (1993) Handbook of Flow Cytometry Methods  
Wiley-Liss, New York, NY.

## II. Cell culture and tissue samples

Adult human primary cells including keratinocytes, melanocytes, and dermal fibroblasts are obtained from Clonetics and cultured according to the suppliers

- 5 instructions. For cytokine treatment, cells are cultured with 10 ng/ml hTNF- $\alpha$  plus 3 ng/ml hIL-1 $\beta$  (R&D Systems) in culture medium. Human T cells are purified from PBMCs using a T-cell enrichment column (R&D Systems) according to the manufacturers instructions.

10

## III. Isolation of encoding sequences

The human, mouse, or rat MIP-3 $\alpha$  sequence is readily available. See Table 1 and GenBank. Appropriate PCR primers or hybridization probes can be selected.

15

Similarly, the human CCR6, or others, can be readily isolated. See Table 2 and GenBank.

## IV. Distribution Analysis

- For Southern blotting, 5  $\mu$ g of each cDNA library is  
20 digested with the appropriate restriction enzymes to release the insert, subjected to gel electrophoresis, and transferred to Hybond-N<sup>+</sup> membrane. For Northern blotting all RNAs are isolated using RNazol B (TEL-TEST, Inc.) and analyzed by electrophoresis on a 1% formaldehyde-agarose gel and  
25 transferred to Hybond-N<sup>+</sup> membrane. Northern and Southern blots are hybridized for 16 hr at 65° C with <sup>32</sup>P-labeled probes obtained by randomly priming (Prime-it; Stratagene) the full length inserts from mouse or human MIP-3 $\alpha$  or CCR6 clones. After hybridization, blots are washed at high stringency and  
30 exposed to film.

- The MIP-3 $\alpha$  was identified from a cDNA library made from human monocytes activated with LPS and IFN- $\gamma$ , in the presence of anti-IL-10. See, Rossi, et al. (1997) J. Immunology 158:1033-1036. Message of the chemokine has also been  
35 detected in pancreatic islet cells, fetal lung, and hepatic

HEPG2 cells, suggesting a physiological role in inflammation or medical conditions in such organs/tissues.

The gene is expressed in HL-60 (promyelocytic leukemia); S3 (HeLa cell); K562 (chronic myelogenous leukemia); MOLT-4  
5 (lymphoblastic leukemia); Raji (Burkitt's lymphoma); SW480 (colorectal adenocarcinoma); A549 (lung carcinoma); and G361 (melanoma) cell lines, as determined by probing on a tissue blot from CLONTECH. Tissue expression gave a positive signal in lymph node, appendix, peripheral blood lymphocytes, fetal  
10 liver, and fetal lung, suggesting a physiological role in inflammation or medical conditions in such organs/tissues; but no detectable signal in spleen, bone marrow, brain, and kidney.

The main transcript appears to be about 1.2 kb, with two  
15 additional transcript sizes in fetal lung RNA. Among the various tissues, transcript sizes of 1.8, 2.7, and 4.2 kb were detected.

Positive signals were also detected in the following cDNA libraries: dendritic cells activated with LPS, but not when  
20 activated with GM-CSF and IL-4; monocytes treated with LPS, IFN- $\gamma$ , and anti-IL-10, but not when treated with LPS, IFN- $\gamma$ , and IL-10; and activated PBMC.

These expression data implicate this chemokine in inflammatory responses upon cell activation. The lymph nodes,  
25 appendix, and PBL are sites where inflammatory processes take place. The MIP-3 $\alpha$  may exert its effects on monocytes and cells involved in inflammatory events. Other structural features implicate this chemokine in eosinophil and lung physiology, e.g., asthma indications. Thus, an antagonist of  
30 the chemokine, e.g., an antibody, may be important for treatment of asthmatic conditions. Also, IL-10 appears to inhibit MIP-3 $\alpha$  expression.

The human MIP-3 $\alpha$  is a ligand for the CCR6. Thus, a positive control exists for the Ca<sup>++</sup> flux assay for that  
35 receptor. This allows for the further screening of agonist ligands for the CCR6. Moreover, the CCR6 was isolated from



eosinophil cDNA, and observations have been made that eosinophils migrate to MIP-3 $\alpha$  in vitro. See, e.g., Greaves, et al. (1997) J. Exp. Med. 186:837-844; Liao, et al. (1997) Biochem. Biophys. Res. Commun. 236:212-217; and Liao, et al. (1998) J. Immunol. 162:186-194. These suggest that the MIP-3 $\alpha$  interaction with the CCR6 is important in recruitment of eosinophils, as occurs with the eotaxin ligand and the CCR3. As such, antagonists of the MIP-3 $\alpha$  interaction with the CCR6 will likely be useful in inhibiting eosinophilia, particularly in the lung, or lung inflammation. These may accompany asthmatic or other pulmonary conditions. The specific upregulation of the pair in inflamed skin suggests a role in skin immunity.

The CCR6 was isolated from a cDNA library made from a dendritic cell cDNA library. It appears to be expressed in certain T cells, spleen cell subsets, NK cells, and other cell populations enriched in dendritic cells, including CD1a<sup>+</sup>, CD14<sup>+</sup>, and CD1Aa<sup>+</sup> cells. It did not give a detectable signal in TF1, Jurkat, MRC5, JY, or U937 cell lines.

Quantitative PCR methods have been applied, e.g., TAQMAN<sup>TM</sup>. High levels of CCR6 cDNA was detected in libraries made from peripheral blood mononuclear cells, resting; T cell, TH0 clone Mot 72, resting; T cell, TH1 clone HY06, anergic; T cell clones, pooled, resting; T cell  $\gamma\delta$  clones, resting; Splenocytes, resting; Splenocytes, activated; B cell EBV lines, resting; NK 20 clones pooled, resting; NK cell clone, NKA6; NK cytotoxic clone, resting; NK cell clone, NK non cytotox; monocytes, LPS,  $\gamma$ IFN, anti-IL-10, 4+16 hr; monocytes, LPS,  $\gamma$ IFN, IL-10, 4+16 hr; DC 70% CD1a<sup>+</sup>, ex CD34<sup>+</sup> GM-CSF, TNF $\alpha$ , activated 1 hr; DC 70% CD1a<sup>+</sup>, ex CD34<sup>+</sup> GM-CSF, TNF $\alpha$ , activated 6 hr; DC 95% CD1a<sup>+</sup>, ex CD34<sup>+</sup> GM-CSF, TNF $\alpha$ , activated 1+6 hr; DC 95% CD14<sup>+</sup>, ex CD34<sup>+</sup> GM-CSF, TNF $\alpha$ , activated 1+6 hr; DC CD1a<sup>+</sup> CD86<sup>+</sup>, ex CD34<sup>+</sup> GM-CSF, TNF $\alpha$ , activated 1+6 hr; DC resting CD34 derived; DC CD41<sup>lo</sup> activated mo derived; DC resting activated mo derived; DC TGF and TGF $\beta$  CD34 derived; lung fetal; gall bladder fetal; small intestine fetal; ovary

fetal; spleen fetal; normal human colon; normal human thyroid; tonsil inflamed; pool of three heavy smoker human lung samples; allergic lung sample; Hashimoto's thyroiditis thyroid sample; and Psoriasis patient skin sample. Intermediate  
5 levels were detected in libraries derived from peripheral blood mononuclear cells, activated; T cell, TH0 clone Mot 72, activated; T cell, TH0 clone Mot 81, resting; T cell, TH0 clone Mot 81, Activated; T cell, TH1 clone HY06, resting; T cell, TH1 clone HY06, activated; B cell line JY, activated; NK  
10 20 clones pooled, activated; NK cell clone, NKB1, pSPORT; NK cell clone, NKB1; DC ex monocytes GM-CSF, IL-4, resting; DC ex monocytes GM-CSF, IL-4, monokine activated 4+16 hr; eosinophils; testes fetal; placenta 28 wk; pool of two normal human lung samples; ulcerative colitis human colon sample;  
15 pool of rheumatoid arthritis samples, human; and normal w.t. monkey colon. Low or undetectable levels were detected in libraries from T cell, TH0 clone Mot 72, anergic; T cell, TH2 clone HY935, resting; T cell, TH2 clone HY935, activated; T cell, TH1 clone TA20-23, resting; T cell, TH1 clone TA20-23,  
20 activated; T cell, TH0 clone B21, resting; T cell, TH0 clone B21, Activated; T cells CD4+, TH2 polarized, activated; T cell lines Jurkat and Hut78, resting; U937 premonocytic line, resting; U937 premonocytic line, activated; monocytes, LPS,  $\gamma$ IFN, anti-IL-10; monocytes, LPS,  $\gamma$ IFN, IL-10; monocytes, LPS,  
25 1 hr; monocytes, LPS, 6 hr; DC 70% CD1a+, ex CD34+ GM-CSF, TNF $\alpha$ , resting; DC ex monocytes GM-CSF, IL-4, resting; DC ex monocytes GM-CSF, IL-4, LPS activated 4+16 hr; kidney fetal; liver fetal; heart fetal; brain fetal; Allergic lung #19; adipose tissue fetal; uterus fetal; normal human skin;  
30 *Pneumocystis carinii* pneumonia lung sample; normal w.t. monkey lung; Ascaris-challenged monkey lung, 24 hr.; and Ascaris-challenged monkey lung, 4 hr.

Being found on dendritic cells, its ligand, including the MIP-3 $\alpha$ , may be important in attracting appropriate cells for  
35 the initiation of an immune response. MIP-3 $\alpha$  has been shown to be a very potent chemoattractant for dendritic cells.

Significant roles of the ligand and receptor in skin physiology are suggested. The receptor may be also present in other cells important in such responses.

## V. Chemotaxis.

Recombinant mouse MIP-3 $\alpha$  is produced in *E. coli* and purified, e.g., as previously described for other chemokines. Hedrick, et al. (1998) Blood 91:4242-4247. Total human T cells in DMEM, pH 6.9, 1% bovine serum albumin, were added to the top chamber of 3  $\mu$ m pore polycarbonate Transwell culture insert (Costar) and incubated with the indicated concentrations of purified chemokine in the bottom chamber for 3 h. The number of migrating cells of each subtype is determined by multi-parameter flow cytometry using fluorochrome conjugated antibodies. A known number of 15  $\mu$ m microsphere beads (Bangs Laboratories, Fishers, IN) is added to each sample before analysis in order to determine the absolute number of migrating cells.

Chemotaxis assays are performed with purified human peripheral-blood T cells and/or skin-homing T cells. Other cell types express the CCR6, e.g., T cells, B cells, DC cells, and granulocyte cells, e.g., neutrophils and/or eosinophils. Recombinant murine MIP-3 $\alpha$  should have effects on the cell types expressing CCR6.

The MIP-3 $\alpha$  and CCR6 expression levels are very low in normal skin samples, but are highly upregulated in inflamed skin tissues.

## 25 VI. Antibody Production

Appropriate mammals are immunized with appropriate amounts, e.g., of MIP-3 $\alpha$  or MIP-3 $\alpha$  gene transfected cells, e.g., intraperitoneally every 2 weeks for 8 weeks. Similar methods may be used to produce antibodies which bind to CCR6, e.g., purified CCR6, polypeptides, or transfected cells expressing the receptor may be used. Typically, rodents are used, though other species should accommodate production of selective and specific antibodies. The final immunization is given intravenously (IV) through the tail vein.

35 Generic polyclonal antibodies may be collected. Alternatively, monoclonal antibodies can be produced. For

example, four days after the IV injection, the spleen is removed and fused to SP2/0 and NS1 cells. HAT resistant hybridomas are selected, e.g., using a protocol designed by Stem Cell Technologies (Vancouver, BC). After 10 days of HAT selection, resistant foci are transferred to 96 well plates and expanded for 3 days. Antibody containing supernatants are analyzed, e.g., by FACS for binding to NIH3T3/surface MIP-3 $\alpha$  transfectants. Many different MIP-3 $\alpha$  mAbs are typically produced. Those antibodies may be isolated and modified, e.g., by labeling or other means as is standard in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY. Methods to conjugate magnetic reagents, toxic entities, labels, attach the antibodies to solid substrates, to sterile filter, etc., are known in the art.

#### VII. Purification of cells

MIP-3 $\alpha$  responsive cells may be identified using the reagents described herein. For example, cells which are chemoattracted towards MIP-3 $\alpha$  may be purified from other cells by collecting those cells which traverse towards MIP-3 $\alpha$ . Such chemotaxis may be to a source of chemokine, or may be across a porous membrane or other substrate. See above, in the microchemotaxis assay.

Alternatively, responsive cells may be identified by expression of the receptor, e.g., CCR6, as provided herein. Thus, antibodies which recognize CCR6 may be used as a positive marker for sorting cells likely to respond to MIP-3 $\alpha$ . Conversely, the marker may be used to deplete CCR6 bearing cells, e.g., by magnetic depletion or toxic conjugates.

Analysis of human samples can be evaluated in a similar manner. A biological sample, e.g., blood, tissue biopsy sample, lung or nasal lavage, skin punch, is obtained from an individual suffering from a skin related disorder. MIP-3 $\alpha$

responsive cell analysis is performed, e.g., by FACS analysis, or similar means.

#### VIII. MIP-3 $\alpha$ Antagonists

5 Various antagonists of MIP-3 $\alpha$  are made available. For example, antibodies against the chemokine itself may block the binding of ligand to its receptor, thereby serving as a direct receptor antagonist. Other antagonists may function by blocking the binding of ligand to receptor, e.g., by binding to the  
10 receptor in a way to preclude the possibility of binding of ligand. Other antagonists, e.g., mutein antagonists or aptamers, may bind to the receptor without signaling, thereby blocking a true agonist from binding. Many of these may serve to block the signal transmitted to target cells, specifically MIP-3 $\alpha$ -  
15 responsive cells. These may be skin cells, including Langerhans, fibroblasts, or keratinocytes.

Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many  
20 different variants at determined positions, e.g., at the positions identified above, and evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which  
25 can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or  
30 across strains or species. Samples from selected individuals are analyzed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent  
35 application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example  
5 only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

## WHAT IS CLAIMED IS:

1. A method of modulating migration of a cell within or to the skin of a mammal, said method comprising administering  
5 to said mammal an effective amount of:

- a) an antagonist of MIP-3 $\alpha$ ;
- b) an agonist of MIP-3 $\alpha$ ;
- c) an antagonist of CCR6; or
- d) an agonist of CCR6.

10 2. The method of Claim 1, wherein said cell is a T cell, B cell, dendritic cell, or dendritic cell precursor.

15 3. The method of Claim 1, wherein said administering is an antagonist of MIP-3 $\alpha$ .

4. The method of Claim 3, wherein said antagonist is selected from:

- a) a mutein of natural MIP-3 $\alpha$ ;
- 20 b) an antibody which neutralizes MIP-3 $\alpha$ ; or
- c) an antibody which binds to CCR6.

25 5. The method of Claim 3, wherein said mammal is subject to a skin disease, including a condition selected from cancer, cancer metastasis, skin transplant, or skin graft.

6. The method of Claim 3, wherein said antagonist is administered in combination with an antibiotic, antifungal, antiviral, or analgesic.

30 7. The method of Claim 3, wherein said antagonist is administered in combination with an immune suppressive therapeutic, a vasodilator, anti-inflammatory drug, growth factor, cytokine, or immune adjuvant.



8. A method of purifying a population of cells, said method comprising contacting said cells with MIP-3 $\alpha$ , thereby resulting in the identification of cells expressing a receptor for said MIP-3 $\alpha$ .

5

9. The method of Claim 8, wherein:

- a) said receptor is CCR6; or
- b) said contacting results in specific migration of said cells to a site for purification.

10

## SEQUENCE LISTING

&lt;110&gt; Schering Corporation

&lt;120&gt; Chemokine Uses; Compositions; Methods

&lt;130&gt; DX0934K

&lt;140&gt;

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&lt;150&gt; 09/224,281

&lt;151&gt; 1999-02-03

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Trp Val Lys Arg Ile Leu His Leu Leu Ser Leu Arg Thr Lys Lys Met  
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<210> 7

<211> 1098

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<213> unknown (surmised Homo sapiens)

<220>

<221> CDS

<222> (1)..(1095)

<220>

<221> misc feature

<222> 579

<223> n may be a, c, g, or t; translated amino acid depends on genetic code

<400> 7

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 1 5 10 15

att act cag ttg att ctg aga tgt tac tgt gct cct tgc agg agg tca 96  
 Ile Thr Gln Leu Ile Leu Arg Cys Tyr Cys Ala Pro Cys Arg Arg Ser  
 20 25 30

ggc agt tct cca ggc tat ttg tac cga att gcc tac tcc ttg atc tgt 144

Gly	Ser	Ser	Pro	Gly	Tyr	Leu	Tyr	Arg	Ile	Ala	Tyr	Ser	Leu	Ile	Cys	
		35					40					45				
gtt	ctt	ggc	ctc	ctg	ggg	aat	att	ctg	gtg	gtg	atc	acc	ttt	gct	ttt	192
Val	Leu	Gly	Leu	Leu	Gly	Asn	Ile	Leu	Val	Val	Ile	Thr	Phe	Ala	Phe	
	50					55					60					
tat	aag	aag	gcc	agg	tct	atg	aca	gac	gtc	tat	ctc	ttg	aac	atg	gcc	240
Tyr	Lys	Lys	Ala	Arg	Ser	Met	Thr	Asp	Val	Tyr	Leu	Leu	Asn	Met	Ala	
	65				70					75					80	
att	gca	gac	atc	ctc	ttt	gtt	ctt	act	ctc	cca	ttc	tgg	gca	gtg	agt	288
Ile	Ala	Asp	Ile	Leu	Phe	Val	Leu	Thr	Leu	Pro	Phe	Trp	Ala	Val	Ser	
				85					90					95		
cat	gcc	act	ggg	gcg	tgg	gtt	ttc	agc	aat	gcc	acg	tgc	aag	ttg	cta	336
His	Ala	Thr	Gly	Ala	Trp	Val	Phe	Ser	Asn	Ala	Thr	Cys	Lys	Leu	Leu	
			100					105					110			
aaa	ggc	atc	tat	gcc	atc	aac	ttt	aac	tgc	ggg	atg	ctg	ctc	ctg	act	384
Lys	Gly	Ile	Tyr	Ala	Ile	Asn	Phe	Asn	Cys	Gly	Met	Leu	Leu	Leu	Thr	
		115					120					125				
tgc	att	agc	atg	gac	cgg	tac	atc	gcc	att	gta	cag	gcg	act	aag	tca	432
Cys	Ile	Ser	Met	Asp	Arg	Tyr	Ile	Ala	Ile	Val	Gln	Ala	Thr	Lys	Ser	
	130					135					140					
ttc	cgg	ctc	cga	tcc	aga	aca	cta	ccg	cgc	agc	aaa	atc	atc	tgc	ctt	480
Phe	Arg	Leu	Arg	Ser	Arg	Thr	Leu	Pro	Arg	Ser	Lys	Ile	Ile	Cys	Leu	
	145				150					155					160	
gtt	gtg	tgg	ggg	ctg	tca	gtc	atc	atc	tcc	agc	tca	act	ttt	gtc	ttc	528
Val	Val	Trp	Gly	Leu	Ser	Val	Ile	Ile	Ser	Ser	Ser	Thr	Phe	Val	Phe	
				165					170					175		
aac	caa	aaa	tac	aac	acc	caa	ggc	agc	gat	gtc	tgt	gaa	ccc	aag	tac	576
Asn	Gln	Lys	Tyr	Asn	Thr	Gln	Gly	Ser	Asp	Val	Cys	Glu	Pro	Lys	Tyr	
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can	act	gtc	tcg	gag	ccc	atc	agg	tgg	aag	ctg	ctg	atg	ttg	ggg	ctt	624
Xaa	Thr	Val	Ser	Glu	Pro	Ile	Arg	Trp	Lys	Leu	Leu	Met	Leu	Gly	Leu	
		195					200					205				
gag	cta	ctc	ttt	ggg	ttc	ttt	atc	cct	ttg	atg	ttc	atg	ata	ttt	tgt	672
Glu	Leu	Leu	Phe	Gly	Phe	Phe	Ile	Pro	Leu	Met	Phe	Met	Ile	Phe	Cys	
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tac	acg	ttc	att	gtc	aaa	acc	ttg	gtg	caa	gct	cag	aat	tct	aaa	agg	720
Tyr	Thr	Phe	Ile	Val	Lys	Thr	Leu	Val	Gln	Ala	Gln	Asn	Ser	Lys	Arg	
	225					230				235					240	
cac	aaa	gcc	atc	cgt	gta	atc	ata	gct	gtg	gtg	ctt	gtg	ttt	ctg	gct	768
His	Lys	Ala	Ile	Arg	Val	Ile	Ile	Ala	Val	Val	Leu	Val	Phe	Leu	Ala	
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tgt	cag	att	cct	cct	aac	atg	gtc	ctg	ctt	gtg	acg	gct	gct	aat	ttg	816
Cys	Gln	Ile	Pro	His	Asn	Met	Val	Leu	Leu	Val	Thr	Ala	Ala	Asn	Leu	
			260					265					270			
ggg	aaa	atg	aac	cga	tcc	tgc	cag	agc	gaa	aag	cta	att	ggc	tat	acg	864
Gly	Lys	Met	Asn	Arg	Ser	Cys	Gln	Ser	Glu	Lys	Leu	Ile	Gly	Tyr	Thr	

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aaa act gtc aca gaa gtc ctg gct ttc ctg cac tgc tgc ctg aac cct			912
Lys Thr Val Thr Glu Val Leu Ala Phe Leu His Cys Cys Leu Asn Pro			
290	295	300	
gtg ctc tac gct ttt att ggg cag aag ttc aga aac tac ttt ctg aag			960
Val Leu Tyr Ala Phe Ile Gly Gln Lys Phe Arg Asn Tyr Phe Leu Lys			
305	310	315	320
atc ttg aag gac ctg tgg tgt gtg aga agg aag tac aag tcc tca ggc			1008
Ile Leu Lys Asp Leu Trp Cys Val Arg Arg Lys Tyr Lys Ser Ser Gly			
	325	330	335
ttc tcc tgt gcc ggg agg tac tca gaa aac att tct cgg cag acc agt			1056
Phe Ser Cys Ala Gly Arg Tyr Ser Glu Asn Ile Ser Arg Gln Thr Ser			
	340	345	350
gag acc gca gat aac gac aat gcg tgc tcc ttc act atg tga			1098
Glu Thr Ala Asp Asn Asp Asn Ala Ser Ser Phe Thr Met			
	355	360	365

&lt;210&gt; 8

&lt;211&gt; 365

&lt;212&gt; PRT

&lt;213&gt; unknown (surmised Homo sapiens)

&lt;400&gt; 8

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Gly Ser Ser Pro Gly Tyr Leu Tyr Arg Ile Ala Tyr Ser Leu Ile Cys	
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Val Leu Gly Leu Leu Gly Asn Ile Leu Val Val Ile Thr Phe Ala Phe	
50 55 60	
Tyr Lys Lys Ala Arg Ser Met Thr Asp Val Tyr Leu Leu Asn Met Ala	
65 70 75 80	
Ile Ala Asp Ile Leu Phe Val Leu Thr Leu Pro Phe Trp Ala Val Ser	
85 90 95	
His Ala Thr Gly Ala Trp Val Phe Ser Asn Ala Thr Cys Lys Leu Leu	
100 105 110	
Lys Gly Ile Tyr Ala Ile Asn Phe Asn Cys Gly Met Leu Leu Leu Thr	
115 120 125	
Cys Ile Ser Met Asp Arg Tyr Ile Ala Ile Val Gln Ala Thr Lys Ser	
130 135 140	
Phe Arg Leu Arg Ser Arg Thr Leu Pro Arg Ser Lys Ile Ile Cys Leu	
145 150 155 160	
Val Val Trp Gly Leu Ser Val Ile Ile Ser Ser Ser Thr Phe Val Phe	
165 170 175	

Asn Gln Lys Tyr Asn Thr Gln Gly Ser Asp Val Cys Glu Pro Lys Tyr  
180 185 190

Xaa Thr Val Ser Glu Pro Ile Arg Trp Lys Leu Leu Met Leu Gly Leu  
195 200 205

Glu Leu Leu Phe Gly Phe Phe Ile Pro Leu Met Phe Met Ile Phe Cys  
210 215 220

Tyr Thr Phe Ile Val Lys Thr Leu Val Gln Ala Gln Asn Ser Lys Arg  
225 230 235 240

His Lys Ala Ile Arg Val Ile Ile Ala Val Val Leu Val Phe Leu Ala  
245 250 255

Cys Gln Ile Pro His Asn Met Val Leu Leu Val Thr Ala Ala Asn Leu  
260 265 270

Gly Lys Met Asn Arg Ser Cys Gln Ser Glu Lys Leu Ile Gly Tyr Thr  
275 280 285

Lys Thr Val Thr Glu Val Leu Ala Phe Leu His Cys Cys Leu Asn Pro  
290 295 300

Val Leu Tyr Ala Phe Ile Gly Gln Lys Phe Arg Asn Tyr Phe Leu Lys  
305 310 315 320

Ile Leu Lys Asp Leu Trp Cys Val Arg Arg Lys Tyr Lys Ser Ser Gly  
325 330 335

Phe Ser Cys Ala Gly Arg Tyr Ser Glu Asn Ile Ser Arg Gln Thr Ser  
340 345 350

Glu Thr Ala Asp Asn Asp Asn Ala Ser Ser Phe Thr Met  
355 360 365



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00511

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/52 C07K14/715 C07K16/24 C07K16/28 C12N5/06  
A61P17/00 A61P35/00 A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 09151 A (SCHERING CORPORATION) 24 February 2000 (2000-02-24) claims 1-20 ---	1-5
X,P	WO 99 20759 A (GENETICS INSTITUTE INC.) 29 April 1999 (1999-04-29) claims 19,23,28,29,40-42 ---	1-5
X	WO 98 01557 A (SCHERING CORPORATION) 15 January 1998 (1998-01-15) claims 1,11 ---	1-5
X	US 5 504 003 A (HAODONG LI ET AL) 2 April 1996 (1996-04-02) column 2, line 34 - line 54 column 9, line 47 - line 58 claims 1-12 --- -/--	1-5

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 July 2000

Date of mailing of the international search report

25/07/2000

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Fax: (+31-70) 340-3016

Authorized officer

Siatou, E

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/00511

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>YOSHIE O ET AL: "NOVEL LYMPHOCYTE-SPECIFIC CC CHEMOKINES AND THEIR RECEPTORS" JOURNAL OF LEUKOCYTE BIOLOGY,US,FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL, vol. 62, no. 5, 1 November 1997 (1997-11-01), pages 634-644, XP002055491 ISSN: 0741-5400 cited in the application abstract</p>	1-9
A	<p>HIESHIMA K ET AL: "MOLECULAR CLONING OF A NOVEL HUMAN CC CHEMOKINE LIVER AND ACTIVATION-REGULATED CHEMOKINE (LARC) EXPRESSES IN LIVER. CHEMOTACTIX ACTIVITY FOR LYMPHOCYTES AND GENE LOCALIZATION ON CHROMOSOME" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 9, 28 February 1997 (1997-02-28), pages 5846-5853, XP002055489 ISSN: 0021-9258 cited in the application abstract</p>	1-9
A	<p>GREAVES D R ET AL: "CCR6, A CC CHEMOKINE RECEPTOR THAT INTERACTS WITH MACROPHAGE INFLAMMATORY PROTEIN 3ALPHA AND IS HIGHLY EXPRESSED IN HUMAN DENDRITIC CELLS" JOURNAL OF EXPERIMENTAL MEDICINE,JP,TOKYO, vol. 186, no. 6, 15 September 1997 (1997-09-15), pages 837-844, XP000199997 ISSN: 0022-1007 cited in the application abstract page 843, right-hand column</p>	1-9
A	<p>ROSSI D L ET AL: "IDENTIFICATION THROUGH BIOINFORMATICS OF TWO NEW MACROPHAGE PROINFLAMMATORY HUMAN CHEMOKINES MIP-3ALPHA AND MIP-3BETA1,2" JOURNAL OF IMMUNOLOGY,US,THE WILLIAMS AND WILKINS CO. BALTIMORE, vol. 158, no. 3, 1 February 1997 (1997-02-01), pages 1033-1036, XP000198409 ISSN: 0022-1767 cited in the application abstract</p>	1-9

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00511

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BABA M ET AL: "IDENTIFICATION OF CCR6, THE SPECIFIC RECEPTOR FOR A NOVEL LYMPHOCYTE-DIRECTED CC CHEMOKINE LARC" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 23, 6 September 1997 (1997-09-06), pages 14893-14898, XP002055488 ISSN: 0021-9258 cited in the application abstract</p> <p>---</p>	1-9
A	<p>POWER C A ET AL: "CLONING AND CHARACTERIZATION OF A SPECIFIC RECEPTOR FOR THE NOVEL CC CHEMOKINE MIP-3ALPHA FROM LUNG DENDRITIC CELLS" JOURNAL OF EXPERIMENTAL MEDICINE,JP,TOKYO, vol. 186, no. 6, 15 September 1997 (1997-09-15), pages 825-835, XP000198408 ISSN: 0022-1007 abstract</p> <p>-----</p>	1-9

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 1-7 refer to the use of agonists or antagonists of MIP-3a or its receptor CCR6 in dermatology. No technical characteristics are given for the agonists or antagonists.

In consequence the scope of claims 1-7 is ambiguous and vague, and their subject matter is neither sufficiently disclosed nor supported (Art. 5 and 6 PCT).

The search has been performed for the parts of the application which are sufficiently disclosed and supported, namely the compounds disclosed in the description

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/00511

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			AU	6444098 A	18-09-1998
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